Supplementary material

SARS-CoV-2 Spike protein binds to bacterial lipopolysaccharide and boosts proinflammatory activity

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Supplementary Figures

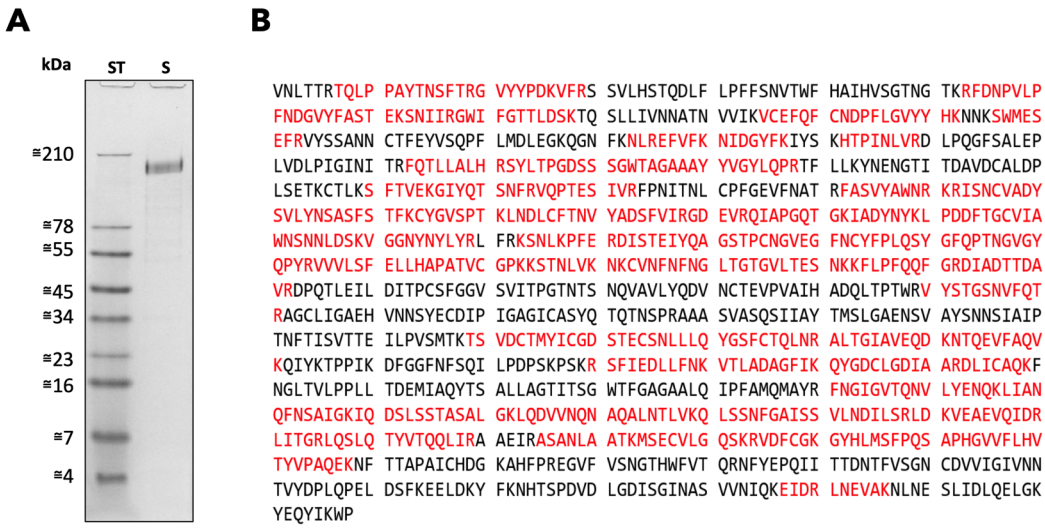

Figure S1 SARS-CoV-2 S protein sequence and endotoxin content. (**A**) 1 µg of SARS-CoV-2 S protein was separated by SDS-PAGE (16.5% Tris-Tricine gel) followed by Coomassie staining. (**B**) LC-MS/MS data were obtained after in gel digestion of SARS-CoV-2 S protein after SDS-PAGE separation. Database analysis confirmed the identity of the recombinant protein to SARS-CoV-2 S protein identifying 56% of the protein sequence as shown in red. Totally 110 peptides correspond to the protein sequence, two other proteins were detected i.e. keratin type II (25% sequence coverage, 14 unique peptides) and keratin type I (9% sequence coverage, 5 unique peptides).

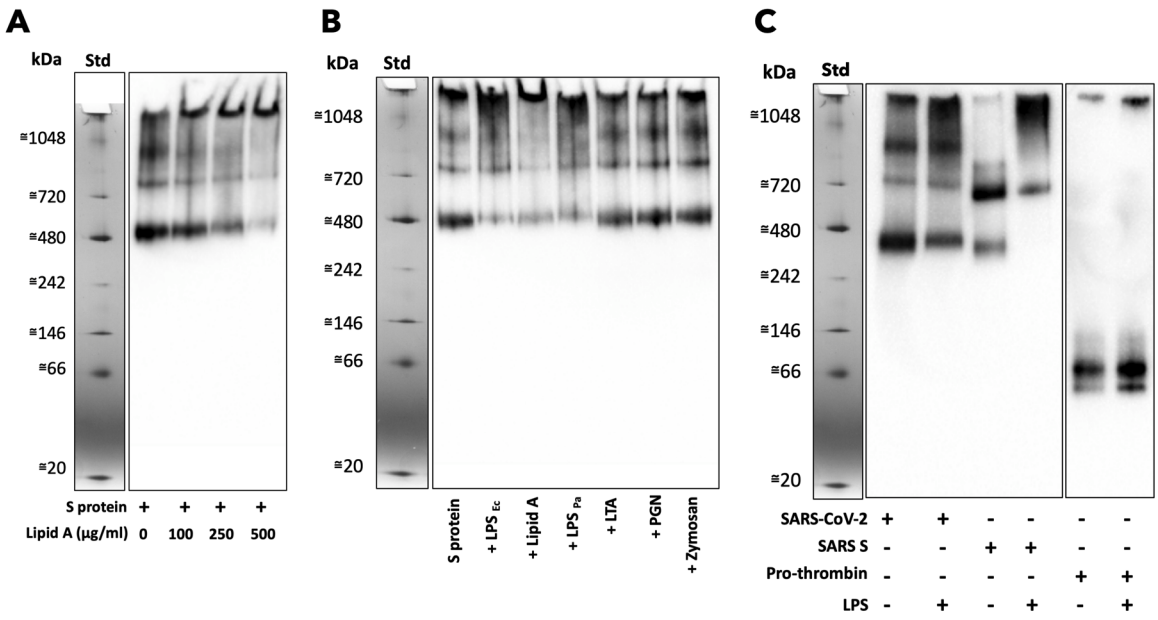

Figure S2 Analysis of binding of SARS-CoV-2 S protein to different TLR ligands or of SARS-CoV S protein to LPS. BN-PAGE followed by Western blotting of (**A**) SARS-CoV-2 S protein incubated with 0-0.5 mg/ml of Lipid A; (**B**) SARS-CoV-2 S protein incubated with 0.25 mg/ml of LPS from *E. coli* (LPS $_{Ec}$), Lipid A, LPS from *P. aeruginosa* (LPS $_{Pa}$), LTA, PGN and zymosan; (**C**) SARS-CoV-2 S protein, SARS-CoV S protein (SARS S) and prothrombin with *E. coli* LPS (0.74 M : 250 µg/ml, protein : LPS).

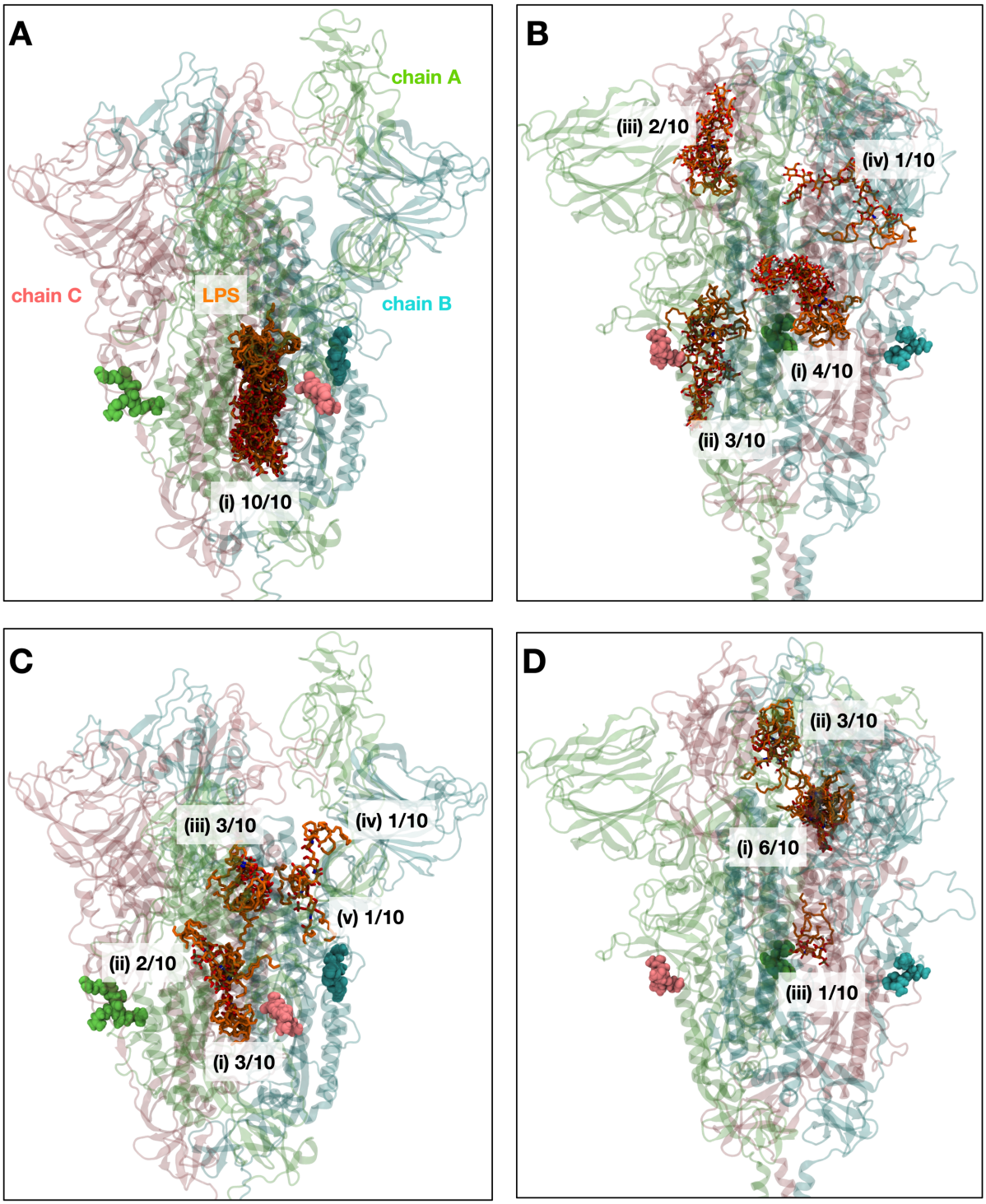

Figure S3 The binding poses generated by flexible docking of *E. coli* LPS and Lipid A on S ECD. (**A**) Docking of LPS (orange; stick representation) onto S ECD in open conformation (green, cyan and pink; cartoon representation). (**B**) Docking of LPS onto S ECD in closed conformation. (**C**) Docking of Lipid A onto S ECD in open conformation. (**D**) Docking of Lipid A onto S ECD in closed conformation. The number of docking poses for each binding site (out of 10 total poses in each docking calculation) are shown. The S1/S2 furin cleavage sites are highlighted in sphere representation.

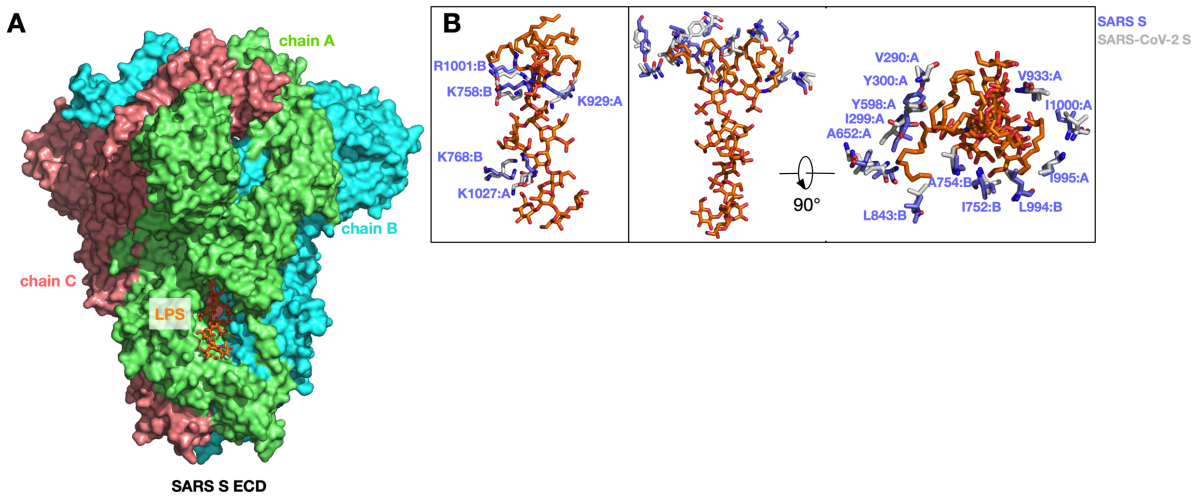

Figure S4 Comparison of SARS-CoV-2 and SARS-CoV S proteins. (**A**) The cryo-EM structure of SARS-CoV S ECD (PDB: 5X58) was aligned to the structure of SARS-CoV-2 S ECD (PDB: 6VSB) and the docked LPS molecule from the latter was mapped to the former. (**B**) The conservation of residues important for binding in the proposed LPS binding site. Residues from SARS-CoV S are shown in purple and labelled, while corresponding residues from SARS-CoV-2 S are shown in white.

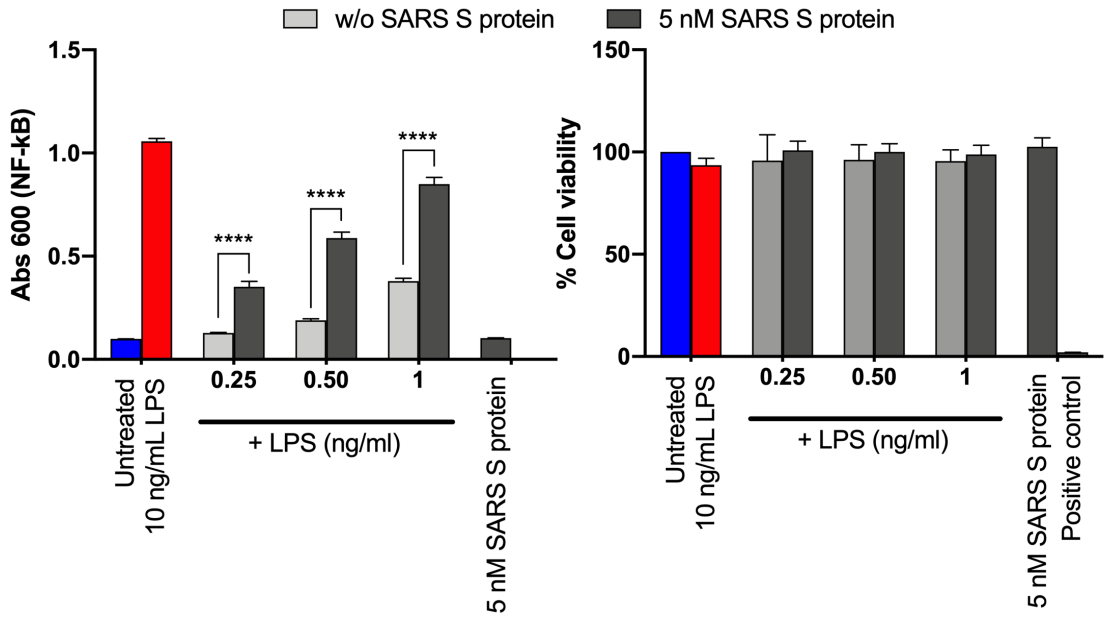

Figure S5 Effects of SARS-CoV S protein on LPS-induced responses in THP-1 cells. THP-1-XBlue-CD14 cells were treated with increasing concentrations of LPS (0.25-1 ng/ml) and 5 nM SARS-CoV S protein (SARS S) then the NF-κB activation was measured. The viability of THP-1 cells after treatment with SARS S protein and LPS was measured by MTT. The mean ± SEM (NF-kB) or SD (MTT assay) values of four independent experiments performed in triplicate are shown (n=4). ****, $P < 0.0001$, determined using two-way ANOVA with Sidak´s multiple comparisons test (NF-kB) or one-way ANOVA with Dunnett's multiple comparison test (MTT assay). na; not analyzed, w/o; without.

Supplementary Table

Table S1. Data for cytokines released from human PBMCs.

	Cytokine/ Chemokine (pg/ml)	Mean ± SEM 0.05 ng/ml LPS -	Mean ± SEM 0.05 ng/ml LPS + 5 nM S protein	*P* value	Mean ± SEM 0.1 ng/ml LPS -	Mean ± SEM 0.1 ng/ml LPS + 5 nM S protein	*P* value
8 h	TNF-α	1289 ± 218.2	2824 ± 311.2	**0.0387**	2204 ± 350.7	3913 ± 392.3	**0.0182**
	IL-6	5328 ± 1354	10651 ± 1876	**0.0336**	9306 ± 1690	14316 ± 1512	**0.0164**
	IL-1β	262.3 ± 63.6	776.4 ± 185.3	0.1747	560 ± 112.7	1326 ± 264.9	0.1445
	IL-8	3935 ± 165.2	4088 ± 96.2	0.7571	4171 ± 36.0	4257 ± 54.5	0.7953
	IFN-β	2.1 ± 0.0	4.5 ± 0.5	0.1034	2.3 ± 0.1	4.4 ± 0.1	**< 0.0001**
	CCL5	557.5 ± 160.1	682.9 ± 115	0.6378	647.1 ± 118.4	647 ± 114.2	> 0.9999
	IL-10	56.28 ± 5.0	138 ± 18.12	0.0926	100.8 ± 10.6	191.4 ± 28.3	0.1999
24 h	TNF-α	1147 ± 141.2	2379 ± 259	**0.0423**	2050 ± 602	3365 ± 413.3	0.2796
	IL-6	7732 ± 1885	14142 ± 1995	**0.0124**	11463 ± 3353	16013 ± 2940	0.0818
	IL-1β	307.7 ± 78.8	992.7 ± 205.1	0.1295	655.1 ± 218.2	1636 ± 313.8	0.1133
	IL-8	4356 ± 63.8	3655 ± 716.1	0.8811	3854 ± 621.1	4403 ± 48.4	0.9247
	IFN-β	2.0 ± 0.1	3.0 ± 0.1	**0.0404**	2.0 ± 0.1	3.1 ± 0.1	0.0563
	CCL5	496 ± 124.6	645.2 ± 132.4	0.3902	565 ± 159.6	716.6 ± 156.5	0.6499
	IL-10	124.4 ± 28.76	817.4 ± 264.6	0.3199	290.4 ± 102	1441 ± 435.4	0.4620

P values were determined using a one-way ANOVA with Tukey's post test. LPS + S protein groups are compared with their respective LPS alone groups (-). *P* values <0.05 are in bold text.